IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Patent Application of Harry R. Davis	: PATENT APPLICATION
Serial No.: 10/057,629	: Group Art Unit: 1617
Filed: January 25, 2002	: Examiner: San Ming R. Hui
For: Use of Substituted Azetidinone Compounds for the Treatment of Sitosterolemia	:Atty. Docket No.: CV01382K

DECLARATION OF HARRY DAVIS, Jr. , Ph.D.

I, Harry Davis, declare and state that:

1. I obtained a Bachelor of Science in Animal and Veterinary Science degree from the University of Maine in 1977.

2. I obtained a Master of Science in Anatomical Pathology degree from George Washington University in 1979.

3. I obtained a Doctorate Degree in Pathology from the University of Chicago in 1982.

4. I am employed by Schering-Plough Research Institute ("Schering") as a Distinguished Research Fellow in the field of Cardiovascular and Metabolic Disease and have been employed in this capacity since 1993 and was previously employed by Schering as a Principal Scientist since November 1987.

5. My duties at Schering have included pharmaceutical drug discovery and basic research in lipid absorption and metabolism and metabolic disease.

6. On a date prior to December 1, 2000, a study was conducted in accordance with my invention, as claimed in the subject patent application. This study was conducted according to my specifications by my coworker, Glen Tetzloff, at my request. Mr. Tetzloff made a record of the preparation in our employer's laboratory notebook marked with the identification "39040," on pages 64-75, and affixed his signature thereto on a date prior to December 1, 2000. The page was witnessed by another coworker on a date prior to December 1, 2000.

7. Attached hereto as "EXHIBIT A" is a true photocopy of pages 64-75 from the laboratory notebook identified as 39040, altered only by obliterating all dates appearing thereon.

As indicated thereon, in vivo activity of SCH 58235 [compound VIII of the present application] in mice was determined by the following procedure:

Male ApoE knockout mice, age 6 weeks, were received from Jackson Laboratory along with age-matched C57BL/J mice. The mice were housed 5 per cage, normal light cycle, normal diet. Twenty-six mice of each variety were weighed and housed, 1 per cage, in suspended wire cages with normal light cycle, normal diet. After three days, the mice were reweighed. Based on body weight, the mice were divided into 5 groups for each type of treatment: Control (corn oil) and Compositions including Compound VIII at 0.3, 1, 3, and 10 mg/kg of body weight per day.

Preparation of Compositions including Compound VIII based on 22g average mouse body weight:

Dosage of Compound VIII (mg/ml/day)	Compound VIII (ml) + corn oil (ml)
10mg/kg/day in 0.1 ml corn oil	2.2mg/ml* 10ml=22 mg in 10ml corn oil
3mg/kg :	3 ml of 10mg/kg + 7 ml corn oil;
1mg/kg :	3 ml of 3mg/kg + 6 ml corn oil;
0.3mg/kg :	2ml of 1mg/kg + 4.67 ml corn oil.

The mice were gavaged using a feeding needle 30 min before receiving ^{14}C-cholesterol (NEN, NEC 018) and ^{3}H-sitosterol (NEN, CUS 030T). The radioactive dose was prepared from:

114 µL ^{3}H-sitosterol stock (1 µCi/µL in ethanol);
1.425 mL ^{14}C-cholesterol stock (40 µCi/mL in ethanol);
5.7 mg cholesterol, Sigma C 8667;
5.7 mg ß-sitosterol, Sigma, S 1270;
The ethanol was removed under N_2;
5.7 ml of corn oil was added, and the mixture was warmed to 60°C;
and shaken for 1hr.

Each 0.1ml dose contained 2 µCi ^{3}H-sitosterol, 0.1 mg cold (non radioactive) sitosterol; 1 µCi ^{14}C-cholesterol, and 0.1 mg cold (non radioactive) cholesterol. Radioactive content was verified: 5 X 10 µl counted in Beckman LSC (liquid simulation counter). Tritiated sitosterol was used as an "unabsorbable" marker to compare to the absorption of [^{14}C]-cholesterol in a mouse fecal isotope ratio cholesterol absorption model.

On the 4th , 5th , and 6th days, feces were collected and stored at -20°C in vials just before dosing with Control or Compound VIII late in the day. Termination of the experiment on the 7th day involved sacrifice by exsanguination, removal and weighing of the liver. 3 X ~250 mg samples of liver were put in vials. The liver samples were digested with 1ml of 1N NaOH at 60° overnight, neutralized with 0.1ml 12N HCl and counted for ^{14}C and ^{3}H. The blood samples were allowed to clot at room temp for 1hr, then centrifuged at 1000G for 15 min. The serum was analyzed for total cholesterol (see Wako CII; see Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC.Enzymatic Determination of Total Serum Cholesterol. Clin. Chem. 1974; 20:470-475, which is incorporated by reference herein) and radioactivity (2 X 50µL). Fecal samples were analyzed for radioactivity by combustion in a Packard Oxidizer followed by Beckman LSC.

9. On a date prior to December 1, 2000, I submitted a Record of Invention Disclosure No. 3669 in accordance with my invention, as claimed in the subject patent application. I affixed my signature thereto on a date prior to December 1, 2000. The page was witnessed by at least one other coworker on a date prior to December 1, 2000.

10. Attached hereto as "EXHIBIT B" is a true photocopy of Record of Invention Disclosure No. 3669, altered only by obliterating all dates appearing thereon.

As indicated thereon, I discovered that that the use of azetidinone compounds (ezetimibe, SCH 58235) inhibits the absorption of the plant sterol sitosterol (see Table 1 below) and that ezetimibe (SCH 58235) may be useful in the treatment of sitosterolemia, by inhibiting the absorption of cholesterol and plant sterols like sitosterol. Wild type mice (C57BL/6J) and mice deficient in apoprotein E (Apo E KO) were found to absorb from 0.15-0.38% of the original [^{3}H]-sitosterol dose administered into their livers. When Compound VIII (SCH 58235) was given, it was found to dose dependently inhibit the absorption and hepatic accumulation of sitosterol.

Table 1.

Effect of Compound VIII on Sitosterol Absorption in Mice				
Mouse strain	Treatment	% of administered dose absorbed of [^{3}H]-sitosterol in liver (total animal liver)		
		average	±sem	p =
C57BL/6J	Control	0.1479	±0.0337	
	Compound VIII 0.3mg/kg	0.1093	±0.0143	
	Compound VIII 1mg/kg	0.0588	±0.0115	(.046)
	Compound VIII 3mg/kg	0.0489	±0.0067	(.024)
	Compound VIII 10mg/kg	0.0552	±0.0151	(.040)
ApoE KO	Control	0.3773	±0.0525	
	Compound VIII 0.3mg/kg	0.1863	±0.0246	0.013
	Compound VIII 1mg/kg	0.1019	±0.0225	0.0019
	Compound VIII 3mg/kg	0.0772	±0.0050	0.0023
	Compound VIII 10mg/kg	0.0780	±0.0179	0.0017
N = 4-6 mice per treatment sem= standard error of mean p= probability				

11. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that those statements were made with the knowledge that willful false statements and the like so made are punishable

by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: July 13, 2004

HARRY DAVIS, Jr., Ph.D.